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Project Title: Inhibition of Fertilizing Ability of Zebra Mussel Sperm

Project Category: Exotic Species

Rank by Organization (if applicable): 0

Total Funding Requested ($): 129,360 **Project Duration:** 2 Years

Abstract:

The economic impact of the zebra mussel invasion has been estimated at $400 million per year based exclusively on water supply disruption to businesses and communities (Schloesser, D. 1995. Encyclopedia of Environmental Biology, vol.2: 533). The impact on sport fishing and ecological consequences (removal of primary production; re-direction of contaminants are only a few examples) have not been documented although hypothetical scenarios have been outlined for some ecosystems (Mackie G. 1991. Hydrobiologia 219:251).

Zebra mussels have an enormous reproductive capacity which is one of the key factors leading to their success in the invasion of the Great Lakes. This research addresses a new technology in which, based on our understanding of the fertilization process of zebra mussel, a range of substances affecting reproductive potential will be examined. This may lead to development of contraceptives to prevent the spread of this species.

The long term objective is to understand zebra mussel reproductive physiology and to identify the potential targets to inhibit sperm fertilizing capability. More specifically, we will concentrate on, (1) characterization of the sperm acrosomal reaction and its regulation by oocyte receptors, (2) acrosomal proteolytic and arylsulfatase activity, (3) characterization of the action of potential anti-proteases and sulfatase inhibitors (N-acetylgalactosamine binding lectins, gossypol, etc.), and (4) exposure of mussel colonies to antifertility factors in situ. There have been basic morphological events established related to fertilization in zebra mussel (Misamore et al. 1996.Mol. Repr. Devel. 43:205; Walker et al. 1996. Can. J. Zool. 74:809), however, detailed study of acrosomal events and antifertility action of gossypol on zebra mussel were not conducted.

Geographic Areas Affected by the Project

States:

- [x] Illinois
- [x] Indiana
- [x] Michigan
- [x] Minnesota
- [x] New York
- [x] Pennsylvania
- [x] Wisconsin
- [x] Ohio

Lakes:

- [ ] Superior
- [ ] Huron
- [ ] Michigan
- [ ] Erie
- [ ] Ontario
- [x] All Lakes

Geographic Initiatives:

- [ ] Greater Chicago
- [ ] NE Ohio
- [ ] NW Indiana
- [ ] SE Michigan
- [ ] Lake St. Clair

Primary Affected Area of Concern: All AOCs

Other Affected Areas of Concern:

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area:

Other Affected Biodiversity Investment Areas:

Problem Statement:

Is there really a need for controlling the zebra mussel invasion? Are there new prospects in the field of contraceptive research that can revolutionize our approach to control reproduction of undesired species in the wild? If there is an evidence of scientific opportunity, is there a climate for development and application of such methods?

The effect of zebra mussel on large ecosystems, such as Lake Erie, might prove that an increase of water clarity paralleled by a return of zooplanktivorous fishes (lake whitefish) is difficult to balance with the disappearance of other species of bivalves, restructuring of the toxicants flow in benthic communities, and physical obstruction to water intakes. However, passage of the 1990 Nonindegenous Aquatic Nuisance Prevention and Control Act sets among others priorities "to develop and carry out environmentally sound control methods." The proximity of mussels to water supplies sets particularly high standards for control methods.

Among the recommended future research and developments is "spawning control measures" (Ludyanskiy et al. 1993. Bioscience 43:533). There is a long list of substances that may block the sperm-oocyte interaction leading to fertilization. These antagonists may lead to inhibition of a particular step of spermatogenesis, suppression of sperm final maturation or induced premature spermatozoa "reaction". It may then be feasible to produce by means of biotechnology such antagonists of fertilization and control the reproductive success of zebra mussel.

Proposed Work Outcome:

Zebra mussel will be collected from Lake Erie and maintained at 12C in a freshwater aquarium system with a 16:8 light:dark cycle. Spawning will be induced thermally or by serotonin (5-HT) (Ram et al. 1993. J. Exp. Zool. 265:587). Preliminary experiments in our laboratory confirmed that animals maintained at cool temperatures and brought to room temperature during exposure to 5HT are capable of gamete release even in December. Therefore, it is possible to do experiments with sperm year-round. We have also optimized conditions of zebra mussel sperm motility (Na, K, pH, temperature) (Ciereszko et al. 2000, Hydrobiologia, in press).

In the first year of study objectives 1 and 3 will be addressed. The acrosin-like activity will be tested as described recently in acrosomal sperm of acipenserid fishes (Ciereszko et al. 1996. Mol. Repr. Dev. 45:72). Alternatively, chymotrypsin-like activity will be tested as discovered recently in lamprey (Petromyzon marinus) sperm (Dabrowski et al. unpublished results). We will optimize the assay (pK substrate concentration, ionic composition) for both proteolytic and sulfatase activities (Dabrowski et al. 1993. Comp. Biochem. Physiol. 104B:717). The most potent acrosin and protease inhibitors (preferably irreversible in the mode of action) will be chosen for further experimentation. Gossypol, for example, is a known acrosin activity inhibitor of mammalian sperm and a general blocker of cell-to-cell communication (Herve et al. 1996. Eur. J.

Pharm. 313:243). Our preliminary results suggest that gossypol inhibits zebra mussel sperm motility at concentrations as low as 6 micromolar, several fold lower than needed to block mammalian sperm activity. Activation and autodegradation of proacrosin/acrosin by carbohydrates and semi-purified oocyte membrane proteins will be examined. We will expose animals during spermatogenesis and those will be induced to release gametes to known male contraceptive, gossypol (Comhaire F. 1994. Human Repr.9: 22).

In conclusion, only with better understanding of normal spermatogenesis and fertilization will it be possible to develop environmentally sound male contraceptive biotechnology for nonindegenous aquatic species.

We will involve graduate students and postdoctoral fellows in this research as part of their training. The results will be disseminated during national and international conferences. The results will be also made available through the Ohio Sea Grant Program publication "Twine Line" and the Ohio State Information Center which prepares fact sheets and press releases.

Project Milestones:	**Dates:**
In vitro experiments with inhibitors	05/2000
Enzyme activities	06/2000
Gamete viability testing	08/2000
In situ experiments during gametogenesis	02/2001
Bioconcentration analysis (gossypol)	06/2001
Enzyme activities	06/2001
Gamete viability testing	06/2001
Project End	05/2002

☐ Project Addresses Environmental Justice

If So, Description of How:

☒ Project Addresses Education/Outreach

If So, Description of How:

We will involve graduate students and postdoctoral fellows in this research as part of their training. The results will be disseminated during national and international conferences. The results will be also made available to the Ohio Sea Grant Program publication "Twine Line" and the Ohio State Information Center which prepares fact sheets and press releases.

Project Budget:

	Federal Share Requested ($)	Applicant's Share ($)
Personnel:	35,485	7,294
Fringe:	11,515	0
Travel:	2,400	0
Equipment:	8,000	2,000
Supplies:	24,000	0
Contracts:	6,600	0
Construction:	0	0
Other:	0	0
Total Direct Costs:	88,000	9,294
Indirect Costs:	41,360	0
Total:	129,360	9,294
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

Description of Collaboration/Community Based Support:

We have already established a collaborative program with a group of researchers (Drs. A. Ciereszko and J. Glogowski) at the Laboratory of Andrology, Polish Academy of Sciences, Olsztyn, Poland. This laboratory has enormous experience in research of the male reproductive system in fishes and invertebrates, specifically with acrosomal sperm and protease inhibitors.